Abstract

The aim of present study was the isolation of different fungal strains for β-galactosidase production. Therefore, 94 soil samples were collected from industrial and agricultural wastelands of Lahore Punjab, Pakistan. These soil samples were used for the isolation of 55 fungal strains. Out of these, 43 fungal strains were primarily screened for β-galactosidase production based on X-gal screening. These strains were subjected to submerged fermentation to analyze their β-galactosidase production ability and maximum production (112.34± 0.23 U/mL/min) was shown by fungal isolate IIB-26. This strain was further subjected to 18S rRNA sequencing and was identified as *A. oryzae*. Optimization of various cultural parameters such as media, pH, temperature, incubation time, carbon sources, nitrogen sources and inoculum size was carried out in order to enhance β-galactosiadse activity. Maximum enzyme production was observed using soya bean meal medium after 120 hours of incubation, at 30°C, pH 4 with 1 percent soya bean meal as carbon and urea as nitrogen source when it was inoculated with 3% inoculum of *A. oryzae*. Ammonium sulfate precipitation followed by anion exchange chromatography was performed in the current study to obtain purity of β-galactosidase which resulted in 90.93% yield and 1.18 purification fold with increase in specific activity of 119.13±0.13 U/mg. The molecular weight of purified β-galactosidase was 55KDa. Enzyme kinetics such as K_m and V_{max} were determined as 0.44mM and 124.86±0.12 U/mL/min, respectively. Thermodynamic studies of the enzyme revealed activation energy (E_a) and enthalpy of activation (ΔH) as 11.39 KJ/mol and 14.60 KJ/mol, respectively. At 55°C and 4.5 pH, the enzyme remained stable and showed maximum activity. The influence of different metal ions and surfactants were checked on the activity of β-galactosidase and its activity was enhanced in the presence of Mg^{+2} (125.12±0.25 U/mL/min), Mn^{+2} (124.54±0.19 U/mL/min) and β-Mercaptoethanol (125.68±0.19 U/mL/min) whereas SDS (78.24±0.21 U/mL/min) and EDTA (89.96±0.19 U/mL/min) inhibited its catalytic activity. From the current study, it is concluded that β-galactosidase can be widely used in the breakdown of lactose into reducing sugars that are used as the main energy reservoirs.